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THE MIDDLE EAST RESPIRATORY SYNDROME CORONAVIRUS (MERS-CoV) – WHAT IS THE RISK? A REVIEW OF RECENT STUDIES*

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Abstract

MERS (Middle East Respiratory Syndrome) is a viral disease of the respiratory system caused by coronaviruses (CoV), which can be contagious to both animals and humans. It was first described in 2012 in Saudi Arabia and very quickly its occurrence was found in European countries. Initially, it was associated with mild changes within the respiratory system, until a new type of virus was isolated in a patient with severe pneumonia and renal failure, who died. The study showed a close relationship between the virus isolated from the patient's cells with HKU4 and HKU5 coronaviruses, previously isolated from bats. The presence of the same virus was found in a patient from Qatar with a similar clinical image. MERS infections, despite relatively low infectivity, are characterized by high mortality (30%). It is believed that the most likely source of the virus for humans are camels. The objective of this article is to review and discuss data on the risk factors of MERS-CoV zoonotic transmission from animals to humans.

Key words: MERS, SARS, betacoronavirus, CoV, camels, bats

MERS (Middle East Respiratory Syndrome) is a new viral disease affects mainly the respiratory system, caused by the virus MERS-CoV (Middle East Respiratory Syndrome Coronavirus). This virus belongs to the family *Coronaviridae,* and its genetic material is a single-stranded RNA of positive polarity.

Initially, it was associated with infections of the respiratory system in animals. The first epidemic of SARS – Severe Acute Respiratory Syndrome broke out in 2002. Since then, within a few months (as of July 31, 2003) 8096 SARS virus infections have been reported, 774 of which caused deaths (WHO, 2004).

After that, mentions of SARS appeared in June 2012. At that time in Saudi Arabia a new previously unknown virus was isolated from a patient with pneumonia and renal failure, who died after 11 days in hospital, due to progressive respira-

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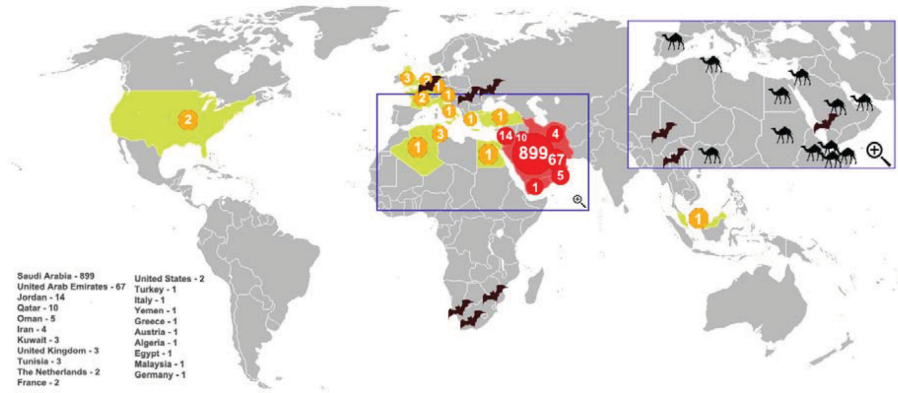
tory failure. Genome sequencing carried out by the Dutch Erasmus Medical Center led to the finding that the virus is phylogenetically closely related to HKU4 and HKU5 coronaviruses, isolated earlier from bats genus *Tylonycteris* and *Pipistrellus*, respectively (Zaki et al., 2012). Three months later, a similar virus was identified in a clinical specimen collected from a patient from Qatar with similar clinical signs (Bermingham et al., 2012).

According to the WHO data of 15 January, 2015, 948 laboratory-confirmed cases of MERS-CoV infections were found all over the world, including at least 349 related deaths (WHO, 2015). The data presented on CoronaMap of 2 March 2015 show 1025 cases of MERS-CoV infections and 399 deaths with 38.9% of case fatality rate (http://coronamap.com/). Figure 1 shows all of the above cases of MERS-CoV infection in humans. It also contains confirmed cases of MERS-CoV infection among camels and bats.



Yellow and green colors – cases of MERS-CoV infection in humans related to travel to the Arabian Peninsula.
Red color – cases of primary MERS-CoV infections.
Bats – cases of MERS-CoV infections in bats.

Figure 1. Map of MERS-CoV infections

Initially, MERS-CoV was called HCoV-EMC/2012, which is an abbreviation of "human betacoronavirus 2c Erasmus Medical Center/2012". This virus is also called EMC/2012 (HCoV-EMC/2012) and has been classified as a new species of the genus *Betacoronavirus*.

MERS-CoV virus

All coronaviruses (CoV) contain genetic material in the form of a single-stranded RNA of positive polarity constructed from 16 to 31 kilobases (kb) with a viral envelope which resembles a crown of surface spikes under electron microscopy, hence the name of the virion.

MERS-CoV virus particle is surrounded by a protein and lipid membrane derived from the host cell, containing a viral glycoprotein M (25–30 kDa), small glycopro-

tein E (9–12 kDa) and glycoprotein S (150–180 kDa). Protein S is in the form of trimers that constitute the typical crown-like peplomers. The shape of MERS-CoV is similar to other coronaviruses, and the viral particle diameter ranges from 70 to 120nm. Genetic material of MERS-CoV is a single-stranded RNA of positive polarity composed of 25.5–32 kb, surrounded by a phosphorylated protein N (50–60 kDa). The nucleocapsid has helical symmetry. Responsible for the interaction of the virus with the host cell and penetration into the cell is the viral glycoprotein S containing two domains: globular S1 located at the N-terminal of the molecule and the transmembrane and intracellular S2 domain. The S1 region of the protein has a highly variable sequence and size, which determines the viral tropism to a specific cell and identification of its specific surface receptor, and in the S2 domain the fusion peptide and two conservative regions HR1 (N-terminal) and HR2 (C-terminal) are located, which form a structure participating in the formation of S protein trimers and in the reaction of membranes fusion during viral entry into the cell. The fusion reaction is preceded by a decomposition of protein S trimer and protrusion of the fusion peptide and its binding from the cell membrane. This process facilitates the cleaving of the S protein into two fragments S1 and S2 by specific proteases such as: cathepsin, human airway trypsin-like protease (HAT), serine proteases 2 (TMPRSS2), which cleave the protein S and initiate conformational changes important for the fusion of the virus envelope with the target cell membrane.

The function of the cellular receptor for MERS-CoV is fulfilled by dipeptidyl peptidase-4 (DPP4) also known as CD26. The structure and shape of DPP4 does not bear resemblance to previously known other receptors for CoV, such as angiotensin-converting enzyme 2 (ACE2) identified for SARS-CoV or aminopeptidase N (APN) for HCoV-229E. All these receptors are expressed on the surface of various cell types, including cells located in the human respiratory tract, and have ectopeptidase activity. However, it appears that this activity does not affect the process of viral entry into the cell. In the glycoprotein S also RBD (receptor binding domain) has been identified, which is responsible for the binding of the viral particle with the receptor.

Six coronaviruses infecting human cells have been identified: HCoV-220E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, SARS-CoV and MERS, as well as various types of CoV affecting also pigs, dogs, cats, rats and domestic birds.

Table 1. Summary of different types of coronaviruses and their hosts

Type of coronavirus	Host
Alpha CoV	human cells: HCoV-220E, HCoV-NL63 dog, pig, cat
Beta CoV	human cells: HCoV-OC43, HCoV-HKU1, HCoV-SARS rat, pig, cow
Gamma CoV	chickens, turkeys
Delta CoV	birds

The construction of virions affects the virus infectivity. In most CoV viruses these proteins are digested intracellularly, resulting in the change of conformation and cleavage of protein into subunits, for example S1 and S2. After the attachment

of the receptor-binding subunit or by protonation occurring after endocytosis, the subunit responsible for the fusion changes its conformation, which results in exposing the hydrophobic fragment of the protein, which penetrates the target membrane. As a result of the change in the conformation to the most energetically stable form, the virion envelope fuses with the membrane of the infected cell (Bosch et al., 2003).

The mechanism of the MERS-CoV infection

The mechanism of viral entry into the cell is non-uniform among different representatives of coronaviruses. Also, different ways of penetration within one species have been described, for example coronaviruses causing SARS can penetrate cells via clathrin-mediated endocytosis or caveolae dependant pathway (Inoue et al., 2007; Wang et al., 2008). Burkard et al. (2014) show that MERS-CoV penetrates through endocytosis and requires no digestion of S protein with lysosomal enzymes. S protein cleavage is carried out with furin (cellular enzyme digesting protein precursors) in the early endosome, which has the effect of fusion of the virion envelope with the endosomal membrane and releasing the virus into the cytoplasm. It has been shown that a furin inhibitor significantly reduces the ability of MERS-CoV to infect the cells. Such a significant response has not been observed in the case of a lysosomal enzymes inhibitor (Burkard et al., 2014). In turn, Qian et al. (2013) have shown that penetration of pseudovirions with S protein is significantly inhibited by lysosomotropic agents (NH_4Cl and bafilomycin, increasing the pH in lysosomes) and inhibitors of cathepsin L (protease acting in lysosomes). Digestion with trypsin of pseudovirions S protein adsorbed to the cell membrane surface at neutral pH, resulted in penetration of the virus into the cell at the cell membrane level and the formation of syncytia (Qian et al., 2013).

Embryonic kidney cells (293T line) were transfected by Qian et al. (2013) with TMPRSS-2, TMPRSS-4 genes (encoding the type II transmembrane serine proteases, subject to the expression in cells of the respiratory system), with the gene encoding S protein and other MERS-CoV viral genes necessary to form pseudovirions. As a result of the transfection, the cells began to produce pseudovirions incapable of further infection (probably because of the prior cleavage of S protein by TMPRSS). However, the formation of large syncytia by kidney cells has been reported. In contrast, cells producing pseudovirions, but not having the gene TMPRSS did not form syncytia, and the pseudovirions created by them were able to infect (Qian et al., 2013). In the endosomal vesicle, a change of the conformation of S1 protein takes place, as well as a fusion of the viral envelope with the membrane of endosomal vesicle and releasing the virus into the cytoplasm.

The authors of these studies conclude that the cleavage of S protein, as in most coronaviruses, is necessary for the fusion of the envelope with the cell membrane. Probably the cleavage occurs during the receptor binding, because the pseudovirion lost its ability to infect when S protein had been cleaved in the cell producing pseudovirions. MERS-CoV is able to penetrate into the cell through endocytosis, but its treatment with lysosomal enzymes is not necessary, because (with the action of suitable proteases) the virus enters the cell at a neutral pH (Qian et al., 2013). This is consistent to some extent with the conclusions of Burkard about membrane fusion

in early endosomes. The possible risk factors are: DPP4 receptor specificity, but also the location of proteases which may cleave S protein in the cells of the infected organism and processing of S protein at the viral maturation processes.

MERS virus infections are characterized by a very high mortality (over 1/3 of infected patients died) and low infectivity. Although transmission of the infectious agent at home has been reported – between family members – as well as in the hospital, it is limited and irregular. However, despite the relatively low infectivity, the virus is still present in the human population. So far, a clear cause of this phenomenon has not been established.

Symptoms of MERS

MERS causes respiratory system diseases of varying severity: from mild to potentially fatal. Most of the deaths involved people over 60 years of age, with low immunity or/and previous diseases. The median age of patients is 47 years; infections were observed between the ages of 9 months to 94 years. The incubation period of the disease is from 2 to 14 days. The symptoms are usually nonspecific, and these are mainly: fever, cough, sore throat, shortness of breath, muscle pain, pain in the chest. Less frequently chills, palpitations and confusion were reported. There can also be symptoms of the digestive system, i.e. diarrhea, vomiting and abdominal pains. However, the virus primarily affects the lower respiratory tract. Patients often require mechanical ventilation. Death usually occurs due to progressive respiratory failure or multiple organ failure. Often acute renal failure was observed. Many secondary cases are characterized by a milder course of the disease or even absence of symptoms – asyptomatic. The rate of positivity among health care workers and family contacts was 1.12% and 3.6%, respectively (Al-Tawfiq and Memish, 2014; Memish et al., 2014 a). Exact pathological changes in human tissues are not known due to the lack of autopsy reports published (van den Brand et al., 2015).

This year were the first reported three cases of neurologic injury associated with MERS-CoV. These patients had severe neurological syndromes, which included an altered level of consciousness ranging from confusion to coma, ataxia and focal motor deficit. Brain MRI revealed striking changes characterized by widespread, bilateral hyperintense lesions on T2-weighted imaging within the white matter and subcortical areas of the frontal, temporal, and parietal lobes, the basal ganglia and corpus callosum. None of the lesions showed gadolinium enhancement (Arabi et al., 2015).

Probably MERS-CoV virus can infect other organs such as lungs, because of the presence of expression of the receptor DPP4 for the virus, on T-cells and in the lung, kidney, placenta, liver, skeletal muscle, heart, brain, endothelium and pancreas. However, this requires further study (Arabi et al., 2015; Abbott et al., 1994; Payne et al., 2014).

Past infections

MERS-CoV infections occur mainly in the Arabian Peninsula. Cases of MERS have been reported in: Saudi Arabia, United Arab Emirates, Qatar, Oman, Jordan, Kuwait, Yemen, Lebanon and Iran. A smaller number of cases is associated with traveling to these places. Such infections were reported among others in citizens of

United Kingdom, France, Italy, Greece, the Netherlands, Germany, Austria and Turkey, as well as the United States of America (CDC, 2014; http://coronamap.com/). Poland remains a country free from MERS.

Nosocomial infections

The first outbreak associated with nosocomial infection took place in Jordan in the city of Az-Zarka (English: Zarqa) in April 2012. This event involved 11 patients, 8 of which were employed in health care (1 fatal case). Initially, the cause of these infections was not known (Hijavi et al., 2013). After publishing information about the MERS virus, additional serological tests were carried out. They showed that out of 124 people associated with this case, 9 had been in contact with the MERS virus (Al-Abdallat et al., 2014). Another outbreak in Jordan in the following year took place in the city of Al-Hasa. The first case of the spread of the virus was linked to a private hospital, and then the disease took over other medical facilities (Assiri et al., 2013). Another outbreak of the disease was recorded in April 2014 in the city of Jeddah. Most infections occurred in a hospital environment, at least in 25% of cases the disease affected health care employees (Al-Tawfiq and Memish, 2014). Between April 11 and June 9, 402 cases in the world were reported (WHO, 2014 b). Only in April 2014 the number of reported cases exceeded the number of all the cases from the previous two years (WHO, 2014 c, d).

The above data shows that MERS appears every year around April, which may indicate seasonality of this type of infection. This phenomenon may be due to increased transmission of the infectious agent from animal reservoirs, but may also be related to the human factor (CIDRAP, 2014).

Intra-community infections

Another group of incidences are intra-community infections. They mainly occur in a family environment. Intra-family infections are likely to be transmitted via droplet route and by direct contact. Another possibility of infection of people from the same family may be contact with similar environmental factors, which can be a source of infection (Al-Tawfiq and Memish, 2014).

Infections associated with travel

Most cases of MERS from outside Saudi Arabia and the surrounding area are secondary cases directly associated with the original case located in the outbreak. According to the ECDC data of April 30, 2014, 10 cases of MERS were reported in Europe, including 2 deaths (one death was reported in Germany) (ECDC, 2014). In both cases, it concerned pilgrims who came to Germany from Mecca and Medina in the United Kingdom of Saudi Arabia (Fanoy et al., 2014). In the first case, as it was established – the infection could be the result of a visit to a medical facility, which usually is the most likely site of infection, but in the second case – after definitive exclusion of direct transmission of the disease from camels or consumption of infected food – the cause remains unclear.

This has caused great concern about the possibility of mass, global spread of the virus as a result of annual pilgrimages to Mecca of the Islam believers (Hajj).

This gathering is considered to be one of the largest in the world. However, research conducted on this subject does not confirm the relationship between the Hajj and the increase in the number of incidences of MERS (Gardner et al., 2014; Memish et al., 2014 b).

Zoonotic infections

Available epidemiological data suggest zoonotic transmission of MERS-CoV virus from animals that are a reservoir of the virus to humans. The most likely source of infection for humans are camels. However, it is believed that the transmission of the virus is done through other intermediate species of animals.

The results of sequencing RNA isolated from bats showed the presence of earlier forms of MERS-CoV virus in bats. The hypothesis that bats may be this intermediate animal species was confirmed by the identification of isolates Essen EMC/2012 MERS-CoV in Egyptian tomb bats *(Taphozous perforatus)* captured in Saudi Arabia (Memish et al., 2013). Moreover, the results of serological tests showed cross-reactions between MERS-CoV antibodies of dromedaries in Oman and antibodies of dromedaries from Canary Islands and Egypt (Nowotny and Kolodziejek, 2014). Unfortunately, studies of cross-reactions occurring between viruses cannot constitute confirmation of interspecies transmission of the virus. Confirmation of the presence of specific types or subtypes of viruses can be viral RNA isolation and sequencing.

High sequence similarity of MERS-CoV virus with viruses HKU4 and 5 isolated from bats suggests that this virus comes from bats. After testing bats in the vicinity of the place of residence of one of the patients, an individual was found whose feces contained genetic material 100% compatible with the material of the virus isolated from a patient with MERS (Memish et al., 2013).

MERS-CoV infections in camels

However, the most likely source of the virus to humans are camels (Nowotny and Kolodziejek, 2014). Both on the Arabian Peninsula and in Africa, a high percentage of seropositive dromedaries was found. Serological tests showed a higher incidence of MERS-CoV infections in adult than in young camels (Al-Tawfiq and Memish, 2014). In addition, young dromedaries ($\leq$ 2 years) had lower viral load than the adult ones, which indicates a higher risk of infection for humans during the reproductive season (spring) when there is a larger number of immunoincompetent camels.

A comparative study of viral sequences isolated from patients with MERS and camels was carried out by Azhar et al. (2014). He showed that in the patient who died of MERS, in samples taken from the nose and in corresponding samples from his nine camels, MERS-CoV RNA was found. MERS-CoV virus was isolated at the same time from the patient and the camels he kept. Complete viral genome sequencing of both isolates confirmed the compatibility of the analyzed sequences.

Similar results were obtained when conducting serological tests: pseudoparticle neutralization (ppNT), microneutralization (MNT) and ELISA for the presence of anti-MERS-CoV antibodies in camels sera acquired from the United Arab Emir-

ates in 2005 (Alexandersen et al., 2014) and the area of Egypt and Saudi Arabia in 2010–2013 (Hemida et al., 2013). In the seroepidemiological study of domestic livestock (sheep, goats, cattle, chicken) and dromedary camels from Saudi Arabia, it was shown that only dromedary camels have evidence of seropositivity to Middle East Respiratory Syndrome coronavirus (MERS-CoV), suggesting an infection with a MERS-CoV-like virus. Tested dromedary camels had a high prevalence of MERS-CoV antibodies (Hemida et al., 2014). There was no evidence of the presence of anti-MERS-CoV antibodies in camels in Australia (Hemida et al., 2014). Studies of archival serum samples extracted from the blood of camels from the camels exporting countries – mainly Sudan and Somalia, gathered over the past 30 years, have shown the presence of neutralizing antibodies MERS-CoV in 81% of the samples. This suggests a long-term circulation of the virus among these animals (Müller et al., 2014).

Also, PCR tests in camels from the areas of Saudi Arabia (in 1993) and Qatar have shown positive results. Interestingly, MERS virus detected in camels in 2013 in Oman and Qatar was slightly different in the tested animals, but to a large extent was similar to the virus in humans from the same geographical area (Nowotny and Kolodziejek, 2013).

Experimental studies show that the camels infected with MERS-CoV isolates harvested from humans showed symptoms of respiratory system infections. The symptoms were mild, however, in all animals a high viral load was observed. It was identified in the serum exudate from the upper respiratory tract for up to 7 days after the inoculation and the presence of viral RNA up to 35 days after infection. The study of the method of the disease spread and affinity to the cells of the upper respiratory tract in dromedaries may help to explain the absence of systemic symptoms of the disease in naturally infected camels and the way of effective transmission from camels to camels and from camels to humans (Adney et al., 2014). At the same time, it cannot be ruled out that camels are the natural reservoir of the virus (Nowotny and Kolodziejek, 2014). In dromedaries from the area of Saudi Arabia, presence of more than one genomic variant of MERS-CoV was found (Briese et al., 2014). The study conducted in Saudi Arabia (Memish et al., 2012) among people working every day with camels has shown that infection with MERS-CoV is not very common in people who take care of dromedaries. In July 2012, material obtained from 300 people having contact with camels and from 50 people not having contact with the animals was tested. The serum was tested serologically with the MERS-CoV spike pseudoparticle neutralization test (ppNT). None of the tested serums gave a positive result.

So far it has not been clearly established how the virus can spread to humans from camels. The most probable seems to be the aerogenic route of infection from camels to humans (Nowotny and Kolodziejek, 2013; Briese et al., 2014). However, there are suppositions that the source of infection could also be camel milk. MERS-CoV virus RNA was detected in the milk of 5 out of 33 tested subjects in Qatar (Reusken et al., 2014). According to the WHO, in several recent cases of MERS, patients consumed raw camel milk. However, there are also many cases of infection in which patients do not have any contact with sick animals (WHO, 2014 a), which may suggest alternative sources of infection.

Among the potential reservoirs of the coronavirus responsible for the Middle East Respiratory Syndrome also farm animals such as cattle, sheep, goats and chickens were considered. Studies in Saudi Arabia have not revealed the presence of anti-MERS-CoV antibodies in the tested sheep (n=100), goats (n=45), cattle (n=50) and chickens (n=240). There were also no cross-reactions between bovine coronaviruses (BcoV) and MERS-CoV, while in many dromedaries both anti-BCoV and MERS-CoV antibodies were present (Hemida et al., 2013). Similar results were obtained by Reusken et al. (2013 a, b). All of the tested animals (goats, sheep and cattle) were negative for anti-MERS-coronavirus (MERS-CoV) antibodies. The tests were performed using microarray technology and virus protein neutralization assays. Serum of the tested dromedaries showed positive results of the neutralization of anti-MERS-CoV (Reusken et al., 2013 a, b).

Coronavirus infections of European bats

In 2009–2011 in Germany, the Netherlands, Ukraine and Romania the fecal specimens of 272 bats from 4 species of the genus *Pipistrellus* (*P. kuhlii, P. nathusii, P. pipistrellus, P. pygmaeus)* were screened for betacoronaviruses. CoV was detected by using nested reverse transcription PCR (RT-PCR) targeting the RNA-dependent RNA polymerase (RdRp) gene (Figure 1). Analysis of RdRps genes in general provides crucial information about virus survival through replication, but also about genome variability and evolution. The RdRps share multiple sequence motifs that are very conserved and used for sequences comparison in taxonomic classification. In the study, authors sequenced the 398-bp CoV RdRp and used RdRp-grouping units (RGU) in taxonomic classification (Annan et al., 2013). The previous study of a partial RdRp fragment sequencing of *P. pipistrellus* CoV of bat feces in the Netherlands colonies has shown genetic material of the virus closely related to MERS-CoV termed VM314 (Reusken et al., 2010). The study revealed the presence of similar sequences in 40 (14.7%) of the 272 examined *P. pipistrellus*, *P. nathusii*, and *P. pygmaeus* bats from the Netherlands, Romania, and Ukraine (Figure 1). The VM314-associated *Pipistrellus* bat betacoronaviruses differed from EMC/2012 by 1.8%. The difference between EMC/2012 and HKU5 was 5.5%–5.9% (Annan et al., 2013).

Drexler et al. (2011) investigated the breeding colony of bats in the attic of a private apartment in the western part of Germany (Figure 1). During three years of work (research began in 2008), from the tested faces samples of the bats, they isolated viral genetic material: coronavirus (1 type), astroviruses (6 types) and 1 novel type of adenovirus (Drexler et al., 2011). Studies of other authors show that young bats and lactating females have a greater chance of being carriers of coronaviruses (Gloza-Rausch et al., 2008; Annan et al., 2013). The authors suggest that breeding colonies of bats living in moderate climates may be a place for the proliferation of coronaviruses. Conducted studies confirm the relationship between the births of bats and the proliferation of the virus in the colony. The study results are shown in simplified form in the following table (Drexler et al., 2011).

Table 2. The relationship between the date of bats births and the results of coronaviruses detection

Date of sampling	Special event	Number of samples in which coronaviruses were found (%)
2008, May 8	achieving full size of the colony	77.5
2008, May 30		22.7
2008, June 20		10
2008, July 10	postpartum period (approx. 1 month after birth)	100
2008, July 31		100
2009, May 27	incomplete data from this year, the colony left the nest	22.5
2009, June 26		72.9
2010, May 11	bats return to the nest	100
2010, May 26		50
2010, June 17		20.4
2010, July 8	postpartum period	97.5
2010, June 23		97.5

The authors note that adenoviruses (as representatives of DNA viruses) do not show such a relationship, most probably because of their greater stability in infected tissues. Persistence of adenoviral infection between individuals means that they do not require continuous transmission in the population. On the other hand, coronaviruses (as RNA viruses exposed to a higher frequency of errors during the genome replication) require continuous transmission and amplification in the population (Drexler et al., 2011).

An additional risk are carnivores that can get into breeding colonies of bats and feed on dead, infected fetuses. For this reason, it is necessary to isolate pet dogs and cats from potential contact with bats (Drexler et al., 2011).

The increase in the number of cases of MERS-CoV infections increases each year around April. This fact raises suspicions that the seasonality of the disease is related to the mechanism of proliferation of coronaviruses in breeding colonies of bats and/or reproductive period of camels.

Experimental infection of animals

Experimental animal studies may be extremely useful in studies of infectivity and the role of risk factors for the development of MERS. There have been many attempts of infecting animals of different species with MERS. These tests involved, among others: rhesus macaques *(Macaca mulatta),* common marmosets *(Callithrix jacchus),* ferrets *(Mustela putorius),* mice *(Mus muris),* as well as Syrian hamsters *(Mesocricetus auratus)* and rabbits *(Oryctolagus cuniculus)*.

Rhesus macaques were infected intratracheally and via various other infection routes. The infections were mild. Two days after infection, the macaques showed transient symptoms of the respiratory system, increased respiratory rate and cough. A rise in body temperature was also recorded. Up to two days after infection, the number of white blood cells and neutrophils in the blood increased. After this time, the number of lymphocytes decreased. On the third day the lungs were congested, and histopathological examination revealed mild multifocal interstitial pneumonia.

It was characterized by thickened interalveolar septa, edema, presence of fibrin, infiltration of small number of macrophages and neutrophils. In the lumen of alveoli macrophages, neutrophils, multinucleated giant cells, fibrin, and sloughed epithelial cells could be seen.

On the sixth day after infection, type II pneumocyte hyperplasia with alveolar pulmonary edema occurred. The presence of viral RNA in type I and II pneumocytes and intra-alveolar macrophages was noted. In rhesus macaques infected intratracheally, the virus was present in the lungs, but not in the upper parts of the respiratory system. There was no evidence of its presence in other organs. In monkeys infected via many routes, the virus was present primarily in the nasal swab on the first and third day after infection, but also in other parts of the respiratory system. Its presence in the bronchoalveolar lavage was noted. Elevated levels of blood proinflammatory cytokines and chemokines capable of inducing chemotaxis and activation of neutrophils were reported.

In marmosets infected with a high dose of MERS-CoV, the disease was more acute than in rhesus macaques. From the first day after infection an increased respiratory rate, and loss of appetite was reported. From the third to sixth day the symptoms were more severe, marmosets had difficulty breathing. Several animals had to be euthanized before the experiment was completed. There were no significant changes in the blood of the animals, apart from hypoproteinemia. The lungs of macaques were heavier and locally hardened. Histopathological examination revealed multifocal pneumonia (moderate to severe) centered around terminal bronchioles and adjacent alveoli.

Experimental infections with MERS-CoV in other animals usually caused mild symptoms. It has been shown that the MERS-CoV virus is not infectious for wild mice, ferrets, Syrian hamsters and guinea pigs (van den Brand et al., 2015).

Cellular risk factors of MERS-CoV infection

Cellular CD26 protein, thanks to which the virus enters the cell, may be a potential risk factor of MERS-CoV infection. CD26 protein, also known as DPP4 (dipeptidyl peptidase 4) (Raj, 2013) performs many functions in eukaryotic organisms, it is among others involved in T-cell activation and digestion of proline-containing peptides. It is expressed in the pancreas and intestine (MEROPS), which may possibly explain the symptoms of the gastrointestinal tract. They can also be found on the surface of the respiratory system cells. Interestingly, CD26 also uses HKU4 virus as a receptor (Yang et al., 2014). In laboratory conditions, it has been shown that cells of some bat species are susceptible to viral infection, and their susceptibility is dependent on the CD26 protein expression on the cell membrane surface. In addition, a permanent bat cells infection was obtained through serial weekly passaging of the virus (the studies were conducted for 9 weeks, on the 63rd day the virus was still present in the cells). It is believed that the right reservoir of the infectious agent are bats, and camels play an intermediate role in the transmission of infection to humans (Cai et al., 2014).

Mutations within genetic material, including in ORF1ab and Spike (S) gene, may have an impact on the adaptation of animal viruses for human transmissions.

Memish et al., in their publication in 2013, when studying a short fragment (182 nucleotides in length) of coronavirus sequence recovered from a sample from an individual *Taphozous perforatus* have shown that this fragment of nucleotides is identical with the same of the MERS-CoV genome sequenced from this patient (referred to as EMC-2012).

Although the sequence is identical to EMC-2012, there is 1 silent transition at position 129 (in the fragment). The bat and the EMC-2012 virus have a 'C' and all the others have a 'T'. The other old virus sequence, Jordan-N3-2012, also has a 'T' at this site, but also a 'T' at position 162 where all the other viruses have a 'C' (http://epidemic.bio.ed.ac.uk/bat_mers-cov).

The research conducted on fecal material of bats of the species *Neoromicia zuluensis* from South Africa has shown fragments of RdRp identical to human MERS-CoV. This study presents nucleotide sequence analysis of a highly conserved region of the coronavirus genome. The analysis was performed on nucleotide sequences in positions 14544–15359 of the ORF1ab gene of MERS-CoV. The study also has a shorter fragment analysis (249 nucleotides) that spans the end of the spike (S) gene and into the NS3a gene in the positions 25099–25347 of MERS-CoV genome (Ithete et al., 2013). The study has shown only one amino acid difference between the new bat virus and MERS-CoV, but 55 nucleotide changes on the RdRp gene region. Between the most divergent pair of MERS-CoV there were only 2 nucleotide differences identified and no amino acid differences. For the spike/NS3a gene fragment the overall nucleotide rate is higher in this fragment, too, once corrected for the sequence length (http://epidemic.bio.ed.ac.uk/mers-cov_reservoir).

To conclude, the analysis of mutations has shown single substitutions in the most polymorphic codons of ORF1ab gene of MERS-CoV. Regardless, substitutions of Spike gene seem to have most significance. Spike is an important host-receptor binding protein for the virus, and hence the repeated non-synonymous substitutions on the same Spike gene codon may hint at the adaptation for human transmissions. However, such conjecture needs further studies for confirmation. It is also noteworthy that recent studies suggest HCoV2c (EMC) does not require angiotensin-converting enzyme 2 for infection and has broad infectivity across some different mammalian cells, including human airway epithelial cells (Müller et al., 2012; Kindler et al., 2013; http://epidemic.bio.ed.ac.uk/coronavirus_mutations).

Conclusions

Available epidemiological data suggest zoonotic transmission of MERS-CoV virus from animals to humans. The most likely primary source of the MERS-CoV to humans are camels. Another investigated source of novel betacoronaviruse 2c are bats. It has not been demonstrated thus far that other animals may constitute a reservoir of MERS-CoV. Mutations within genes coding important viral proteins, such as ORF1ab and Spike, may have an impact on the ability of MERS-CoV adapting in various host species. So far it has not been clearly established how the virus can spread. The most probable seems to be the aerogenic route of infection from camels to humans. However, there are some cases of infection in which patients do not have any contact with sick animals, which may suggest alternative sources of infection,

for example, the consumption of raw milk. Despite causing human cases for over 2 years, this virus has resolutely stayed geographically constrained to the Arabian Peninsula. According to the ECDC, in the European Union in the near future, more cases of confirmed MERS-CoV infections can be expected. There is also risk of transmission of the infection to our country. According to the WHO, the current situation does not indicate a public health threat of international concern. Therefore, it is not recommended to use restrictions related to trade or traveling to the Middle East or further anti-epidemics actions undertaken by services of the countries in which there have been infections. Poland still remains a country free from Mers-CoV infections. Information on the MERS-CoV infections is constantly monitored all over the world by both WHO and ECDC.

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